106 indicates CDR-3, and amino acids 107 to 117 indicates framework 4.--

B1 cont.

Replace the paragraph beginning at page 11, line 6, with the following rewritten paragraph:

--Sequence ID No. 40 in the Sequence Listing shows gene sequence and amino acid sequence (SEQ ID NO: 64) encoded thereby of the variable part of L chain of MY10 antibody. In Sequence ID No. 40 in the Sequence Listing, amino acids 1 to 23 indicates framework 1, amino acids 24 to 39 indicates CDR-1, amino acids 40 to 54 indicates framework 2, amino acids 55 to 61 indicates CDR-2, amino acids 62 to 93 indicates framework 3, amino acids 94 to 102 indicates CDR-3, and amino acids 103 to 113 indicates framework 4.--

B2

Replace the paragraph beginning at page 11, line 15, with the following rewritten paragraph:

--Sequence ID No. 41 in the Sequence Listing shows gene sequence and amino acid sequence (SEQ ID NO: 65) encoded thereby of the single chain antibody. In Sequence ID No. 41 in the Sequence Listing, amino acids 1 to 39 indicates a sequence containing a signal for secretion from Escherichia coli, amino acids 40 to 156 indicates a variable region of H chain, amino acids 157 to 171 indicates a linker, amino acids 172 to 284 indicates a variable region of L chain, and amino acids 285 to 302 indicates a sequence containing an E-tag.--

B3

Replace the paragraph beginning at page 24, line 4,

with the following rewritten paragraph:

--In the case of single chain antibodies, the effect of decreasing antigenicity can be expected by reducing the molecular weight. The antibody converted to Fab by treating the antibody with a protease such as papain still contains the mouse constant region. In contrast, the single chain antibody is free of them, so that the antigenicity can be decreased to a very low level. As a method for isolating the single chain antibodies from hybridomas, Recombinant Phage Antibody System kit (Pharmacia) and the like may be utilized. However, where the single chain antibody is toxic to Escherichia coli, a production host, and causes death of Escherichia coli or decomposition of the single chain antibody occurs, kits cannot be used effectively so that great ingenuity is required. The single chain antibody can be prepared by studying inductive vector such as pSE380 plasmid (Invitrogen) or pET24d(+) plasmid (Novagen) and bacterial species serving as a host. In production, besides the above-described method, an eukaryotic cell expression system, an insect cell expression system, and a yeast cell expression system can be utilized efficaciously. As the linker which links H chain and L chain, 15 amino acid residues made of three repetitions of (Gly-Gly-Gly-Gly-Ser) (piece of SEQ ID NO: 9) was used. However, linkers not limited to this sequence may be used.--

Replace the paragraph beginning at page 40, line 23, with the following rewritten paragraph:

--The amino acid sequences of single chain antibodies are indicated by Sequence ID Nos. 9 and 10 in the Sequence Listing. Also, examples of nucleic acid sequence encoding them are shown together with the amino acid sequences. In (SEQ ID NOS 9 and 59) in the Sequence Listing, the amino acids 1 to 22 indicates a sequence containing a signal for secretion from Escherichia coli, amino acids 23 to 133 indicates a variable region of L chain, amino acids 134 to 148 indicates a linker, amino acids 149 to 266 indicates a variable region of H chain, and amino acids 267 to 305 indicates a sequence containing FLAG-tag (amino acids 270 to 277), c-myc-tag (amino acids 281 to 290), and (piece of SEQ ID NO: 9) His×6-tag (amino acids 296 to 301)). In (SEQ ID NOS 10 and 60) in the Sequence Listing, the amino acids 1 to 22 indicates a sequence containing a signal for secretion from Escherichia coli, amino acids 23 to 140 indicates a variable region of H chain, amino acids 141 to 155 indicates a linker, amino acids 156 to 266 indicates a variable region of L chain, and amino acids 267 to 305 indicates a sequence containing FLAG-tag (amino acids 270 to 277), c-myc-tag (amino acids 281 to 290), and (piece of SEQ ID NO: 10) His×6-tag (amino acids 296 to 301)).--

B5

Replace the paragraph beginning at page 41, line 17, with the following rewritten paragraph:

--A single chain antibody can be expressed in Escherichia coli cells by deleting the amino acids 1 to 20 of the

B6

amino acid sequences described under (SEQ ID NOS 9, 59, 10 and 60) in the Sequence Listing. The amino acids 267 to 305 of the amino acid sequences described under (SEQ ID NOS 9, 59, 10 and 60) in the Sequence Listing is a sequence for detecting and purifying single chain antibodies and it can be deleted or replaced by any sequence. Preparation of a vector containing a signal for secretion from Escherichia coli and a sequence for detection and purification is shown as Reference Example 2. The linker in the amino acid sequence (SEQ ID NO: 59) 134 to 148 described under Sequence ID No. 9 in the Sequence Listing and the linker in the amino acid sequence (SEQ ID NO: 60) 141 to 155 described under Sequence ID No. 10 in the Sequence Listing may be replaced by any sequence as far as the steric configuration of the variable regions of L chain and H chain are retained substantially the same as the 4H5 antibody. The Lys residues at the 305 positions of the amino acid sequences under SEQ ID NOS 9, 59, 10 and 60 in the Sequence Listing functions effectively so as to align the orientation of single chain antibody molecules where the antibody is immobilized to the water-insoluble carrier. In order to increase the efficiency of immobilization, plural Lys residues may be introduced. It is also effective to introduce Cys residues. The above-described modifications can be practiced with ease by genetic engineering techniques.--

B6

Replace the paragraph beginning at page 61, line 8, with the following rewritten paragraph:

--The gene sequence of 4H5 antibody H chain variable region (whole length) determined by the above operations is shown by Sequence ID No. 37 together with its amino acid sequence (SEQ ID NO: 61) and the gene sequence of L chain variable region (whole length) determined by the above operations is shown by Sequence ID No. 38 together with its amino acid sequence (SEQ ID NO: 62).--

B1

Replace the paragraph beginning at page 69, line 5, with the following rewritten paragraph:

--To incorporate antibody genes into ScFv antibody producing vector, the genes described under (SEQ ID NOS 37, 61, 38 and 62) in the Sequence Listing obtained in Example 2 were amplified using primers containing a restriction enzyme sequence and a linker sequence by PCR method. As the primer for L chain, Sequence ID No. 27 in the Sequence Listing (5'AGCCGGCCATGGCCGACATTGTGCTGACCCAATCTCCA3') and Sequence ID No. 28 in the Sequence Listing (5'CTCCGGAGCCACCTCCGCCTGAACCGCCTCCACCTTTGATTTCCAGCTTGGTGCCTCC3') were used while as the primer for H chain, Sequence ID No. 29 in the Sequence Listing (5'CTCCGGAGGTGGCGGATCGCAGGTTCAGCTGCAGCAGTCT3') and Sequence ID No. 30 in the Sequence Listing (5'TGCGGCCGCTGCAGAGACAGTGACCAGAGTC3') were used. The PCR conditions were such that using GeneAmp PCR Reagent Kit with AmpliTaq DNA Polymerase (TaKaRa Shuzo), a cycle of 94°C for 45

B8

seconds, 58°C for 45 seconds, and 72°C for 1 minute was repeated 18 times. The PCR apparatus used was Model name: DNA Thermal Cycler 480 (Perkin Elmer). The amplified fragments were each cloned using TA cloning kit (Invitrogen). The L chains of the genes were digested with restriction enzymes NaeI (TaKaRa Shuzo) and MroI (Toyobo) and the H chains of the genes were digested with restriction enzymes MroI (Toyobo) and NotI (TaKaRa Shuzo) and then electrophoresed in 2% agarose gel. The gel portion containing each DNA fragment was cut out and extracted. For the extraction of DNA fragment from the agarose gel, Gene CleanII Kit (Bio 101) was used.--

Replace the paragraph beginning at page 71, line 9, with the following rewritten paragraph:

--~~To incorporate~~ antibody genes into ScFv antibody producing vector, the genes described under (SEQ ID NOS 37, 61, 38 and 62) in the Sequence Listing obtained in Example 2 were amplified using primers containing a restriction enzyme sequence and a linker sequence by PCR method. As the primer for H chain, Sequence ID No. 31 in the Sequence Listing (5'AGCCGGCCATGGCCCAGGTTCAGCTGCAGCAGTCT3') and Sequence ID No. 32 in the Sequence Listing (5'CTCCGGAGCCACCTCCGCCTGAACCGCCTCCACCTGCAGAGACAGTGACCAGAGTC3') were used while as the primer for L chain, Sequence ID No. 33 in the Sequence Listing (5'CTCCGGAGGTGGCGGATCGGACATTGTGCTGACCCAATCTCCA3') and Sequence ID

No. 34 in the Sequence Listing (5'TGCGGCCGCTTTGATTTCCAGCTTGGTGCCTCC3') were used. The PCR conditions were such that using GeneAmp PCR Reagent Kit with AmpliTaq DNA Polymerase (TaKaRa Shuzo), a cycle of 94°C for 45 seconds, 58°C for 45 seconds, and 72°C for 1 minute was repeated 18 times. The PCR apparatus used was Model name: DNA Thermal Cycler 480 (Perkin Elmer). The amplified fragments were each cloned using TA cloning kit (Invitrogen). The H chains of the genes were digested with restriction enzymes NaeI (TaKaRa Shuzo) and MroI (Toyobo) and the L chains of the genes were digested with restriction enzymes MroI (Toyobo) and NotI (TaKaRa Shuzo) and then electrophoresed in 2% agarose gel. The gel portion containing each DNA fragment was cut out and extracted. For the extraction of DNA fragment from the agarose gel, GeneCleanII Kit (Bio 101) was used.--

Replace the paragraph beginning at page 86, line 4, with the following rewritten paragraph:

--Thereafter, the PCR method was practiced to amplify the target gene. As for the primer, a plurality of candidate sequences that mouse antibody gene cDNA can be synthesize were synthesized with reference to the gene sequence of mouse antibody variable region described in Sequences of Proteins of Immunological Interest, 5th edition, 1991 (published by USA NIH), and PCR method was conducted combining these primers. Genes were amplified from the primers having the nucleic acid sequences

(5'GTCCCAGGATCCTCTGAAGCAGTCAGGCCC3') (SEQ ID NO: 49) and (5'ACAGTGGGCCCGTCGTTTTGGCTGAGGAGA3') (SEQ ID NO: 50) concerning the procurement of H chain and from the primers having the nucleic acid sequences (5'TGTGCCCTCGAGGTGACTCAAACTCCACTCTC3') (SEQ ID NO: 51) and (5'ATGGATACTAGTGGTGCAGCATCAGCCC3') (SEQ ID NO: 52) concerning the procurement of L chain. The amplified gene fragments were cloned using TA cloning kit (Invitrogen). The obtained gene was determined on the nucleotide sequences of the H chain and L chain of the variable region using DNA Sequencer ver. 1.2.0, Model 373 (Applied Biosystems) according to the protocol attached thereto. The labeling reaction was performed using a nucleic acid sequence (5'CTCTTGGAGGAGGGTGCCAG3') (SEQ ID NO: 53) for H chain and a nucleic acid sequence of (5'CCAGATTTCAACTGCTCATCAGA3') (SEQ ID NO: 54) for κ chain as primers and using PRISM Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems). The method was in accordance with the protocol attached to the kit. The staining was performed using a labeling kit of ABI. As a result, the gene fragment obtained from the primer of H chain is shown by Sequence ID No. 39 and the gene fragment obtained from the primer of L chain is shown by Sequence ID No. 40.--

B10 Cont.

Replace the paragraph beginning on page 91, line 11, with the following rewritten paragraph:

--To incorporate antibody genes into ScFv antibody producing vector, the genes obtained in Example 19 were amplified

B11

by a PCR method using primers containing restriction enzyme sequences. The primers used for H chain had nucleic acid sequences (5'GCGGCCCAGCCGGCCATGGCCCAGGTGCAGCTGAAGCAGTCAG3') (SEQ ID NO: 55) and (5'AGACGGTGACCGTGGTGCCTTGGCCCC3') (SEQ ID NO: 56) and those for L chain had nucleic acid sequences of (5'TCGAGCTCACTCAGTCTCCACTCTCCCTGCCT3') (SEQ ID NO: 57) and (SEQ ID NO: 58) (5'CACCTGCGGCCGCCCGTTTCAGCTC3'). The PCR conditions were such that using GeneAmp PCR Reagent Kit with AmpliTaq DNA Polymerase (TaKaRa Shuzo), a cycle of 94°C for 1 minute, 55°C for 1 minute, and 72°C for 2 minutes was repeated 30 times. The PCR apparatus used was Model name: DNA Thermal Cycler 480 (Perkin Elmer). The H chains of the amplified genes were digested with restriction enzymes SfiI (Toyobo) and BstPI (TaKaRa Shuzo) and the L chains of the amplified genes were digested with restriction enzymes SacI (TaKaRa Shuzo) and NotI (TaKaRa Shuzo) and then electrophoresed in 1.5% agarose gel. The gel portions containing each DNA fragment was cut out and extracted. For the extraction of DNA fragment from the agarose gel, GeneCleanII Kit (Funakoshi) was used.--

B11 Cont.

Replace the paragraph beginning at page 102, line 11, with the following rewritten paragraph:

B12

--The pCANMY10 vector prepared in Example 21 was improved to prepare an antibody which can increase bindability to the water-insoluble carrier. Subsequent to the E-tag sequence at the carboxyl terminal of the single chain antibody were